Modulation of activity of neurotrophins

All patent and non-patent references cited in the application, or in the present application, are also hereby incorporated by reference in their entirety.

Field of invention

The present invention relates to compositions which are useful in modulating neurotrophin activity, as well as methods for the preparation and use thereof. Methods are also provided for screening for agents for use in said compositions.

Background of invention

The neurotrophin family

Neurotrophins are dimeric peptide hormones. The first member of the neurotrophin family to be discovered was nerve growth factor (NGF), which plays an important role in processes such as the development of sensory and sympathetic neurons of the peripheral nervous system (Levi-Montalcini, R. and Angeleeti, P.U:, Physiol. Rev. 48, 534-569 (1968)). The next member of the neurotrophin family to be isolated was brain-derived neurotrophic factor (BDNF), also referred to as neurotrophin-2 (NT-2), the sequence of which was published by Leibrock, J. et al. in 1989 (Nature 341, 149-152). In 1990 several groups identified a neurotrophic factor originally called neuronal factor (NF), now referred to as neurotrophin-3 (NT-3) (Ernfors et al., Proc.Natl:Acad.Sci.USA 87, 5454-5458 (1990); Hohn et al., Nature 344, 339; Maisonpierre et al., Science 247, 1446; Rosenthal et al., Neuron 4, 767; Jones and Reichardt, Proc. Natl. Acad. Sci. USA 87, 8060-8064; Kaisho et al., FEBS Lett. 266, 187). Neurotrophins-4 and –5 were then added to the family (Neuron 6, 845-858 (1991); Berkmeier, L. R. et al., Neuron 7, 857-866 (1991); Ip et al., Proc. Natl. Acad. Sci. USA 89, 3060-3064 (1992)).

Receptors for the neurotrophin family

In a similar way to other polypeptide growth factors, neurotrophins affect their target cells through interactions with cell surface receptors. According to current knowledge, neurotrophins bind to two discrete receptor types which can be distinguished

pharmacologically: the Trk and p75 ^{NTR} neurotrophin receptors. p75^{NTR} is a member of the Fas/tumour necrosis factor (TNF) receptor family, and can interact with all the mammalian members of the neurotrophin family with equal affinities (Rodriguez-Tebar et al. 1990, Neuron 4:487-492; Barker and Murphy, 1992, Mol. Cell. Biochem. 100:1-15). Cells expressing TrkA, a tyrosine kinase receptor originally identified as a human oncogene (Mltin-Zanca et al, Nature 319:743-748) bind solely to NGF and exhibit significantly slower dissociation kinetics (Jing et al. 1992, Neurol. 9:1067-1079; Loeb and Greene, 1993, Neuroscience 13:2919-2929). BDNF binds the TrkB receptor only, but NT-3 can bind all three Trk (A, B and C) receptors, with a preference for TrkC. NT-4/5 can bind both TrkA and TrkB (Ip et al. PNAS 89:3060-3064; Klein et al. Neuron 9:947-956). NT-7 does not interact with TrkB or TrkC but can however induce tyrosine phosphorylation of TrkA, indicating a similar receptor specificity as NGF (Nilsson et al., FEBS Lett (1998) Mar 13;424(3):285-90). Recombinant purified NT-6 also has a spectrum of actions similar to NGF but with a lower potency (Gotz et al., Nature (1994) Nov 17;372(6503):266-9).

The neurotrophin family: precursor proteins

The biology of the neurotrophin family is complex: the neurotrophins are synthesised intracellularly as 30-35 kDa precursor proteins, containing a signal peptide and glycosylation sites. During processing precursor proteins are also cleaved at a dibasic cleavage site by the calcium-dependent serine protease furin and other members of the prohormone convertase family, within the Golgi apparatus. The N-terminal part of this cleavage is the mature neurotrophin of 118-120 amino acids and a biologically active 12-14 kDa C-terminal product (Seidah et al, Biochem. J. (1996) 314:951-960).

Clinically relevant roles of the neurotrophin family

Neurotrophins are of clinical interest as they play an important role in neuronal cell survival and differentiation (Thoenen 1991, Trends Neurosci. 14: 165-170; Raffioni et al. 1991, Ann. Rev. Biochem. 62:823-850; Chao, 1992, Neuron 9:583-593; Barbacid 1993, Oncogene 8:2033-2042). Trk receptors transmit signals promoting neuronal survival, whereas p75^{NTR} can induce neuronal apoptosis as well as neuronal survival depending on any co-expression of TrkA (Miller et al., Cell. Mol.

Life Sci. 58:1045-1053 (2001)). Certainly, it has been demonstrated that activation of TrkA receptors can negate the proapoptotic effect of p75 ^{NTR} (Yoon et al., J. Neurosci. (1998) 18:3273-3281).

It is probable that the propeptides of neurotrophins play important biological roles: at least one neurotrophin precursor protein (proNGF) and its proteolytically processed and mature counterpart (NGF) product differentially activate pro- and anti-apoptotic cellular responses through preferential activation of p75 ^{NTR} and TrK receptors, respectively - pro-NGF having enhanced affinity for p75 ^{NTR} receptors and a reduced affinity for Trk receptors relative to the mature forms of NGF. Indeed, it has been demonstrated that pro-NGF induces p75^{NTR}-dependent apoptosis in cultured neurons with minimal activation of TrkA-mediated differentiation or survival (Lee et al., Science (2001), 294:1945-1948).

Furthermore, neurotrophins are of clinical interest as it is known that both up-regulation of neurotrophins and increased p75^{NTR} expression occur under pathological and inflammatory conditions, especially after nerve injury and damage to the vascular system. Indeed, Soilu-Hanninen et al. have demonstrated that the pro-apoptotic functions of p75^{NTR} are directly implicated in injury-induced apoptosis (Soilu-Hanninen et al., J. Neurosci. 19:4824-4838 (1999)). Recently, it was also demonstrated that proNGF induces p75 mediated death of oligodendrocytes following spinal cord injury (Beatty et al., Neuron (2002), vol. 36, pp. 375-386).

It has been hypothesized that the lack of neurotrophic factors is responsible for the degeneration of selective neuronal populations as it occurs in Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis, and that application of corresponding neurotrophic factor might prevent neuronal degeneration [Appel, S. H., "A unifying hypothesis for the cause of amyotrophic lateral sclerosis, parkinsonism, and Alzheimer's disease," Ann. Neurol. 10:499-505 (1981)]. In particular, as NGF is a trophic factor for the population of basal forebrain cholinergic neurons which degenerates in Alzheimer's disease, it has been speculated that NGF may be useful in the treatment of this disease.

Another reason for interest in targeting neurotrophin pathways for therapy is that studies have provided supporting evidence for the involvement of neurotrophins in

depression and antidepressant action (Duman et al. Arch Gen Psychiatry (1997) 54:597-606); for instance infusion of BDNF into the hippocampus has produced an antidepressant effect in two behavioural models of depression (Shirayama et al. (2002), J Neurosci 22(8): 3251-3261).

The Vps10p-domain receptor family

Sortilin (or NTR-3 or GP95) is a type I membrane receptor expressed in a number of tissues, including the brain, spinal cord, testis and skeletal muscle (Petersen et al., J. Biol. Chem., 272:3599-3605 (1997); Herman-Borgmeyer et al., Mol. Brain Res., 65:216-219 (1999)). Sortilin belongs to a family of receptors comprising Sortilin, SorLA (Jacobsen et al., J. Biol. Chem., 271:31379-31383 (1996)), SorCS1, SorCS2 and SorCS3. All the receptors in this family share the structural feature of an approximately 600-amino acid N-terminal domain with a strong resemblance to each of the two domains which constitute the luminal portion of the yeast sorting receptor Vps10p (Marcusson, E.G., et al., Cell, 77:579-586 (1994)). The Vps10p-domain comprises a C-terminal segment containing 10 conserved cysteines and an N-terminal propeptide of 40-80 amino acids.

In Sortilin, the propeptide exhibits high affinity binding to the fully processed receptor. Prevention of propeptide cleavage essentially inhibits ligand binding to Sortilin, indicating that the propeptide sterically hinders ligands from gaining access to their binding sites on the receptor (Petersen et al., EMBO J., 18:595-604, 1999).

Some progress has been made as to an understanding of the role of this family: there is evidence suggesting that Sortilin at least contains YXXΦ and dileucine motifs, conforming to potent signals for Golgi-endosome sorting (Nielsen et al., EMBO 20(9):2180-2190). It is probable that the other members of the family may also fulfil a similar "sorting" function, not least because they all exhibit homology to Vsp10p, the sorting receptor for carboxypeptidase Y (CPY) in yeast. Only a small proportion of Sortilin receptors are present on the cell surface (Mazella et al. J. Biol. Chem. (1998) 273, 26273-26276; Morris et al. J. Biol. Chem. (1998) 273:3582-3587), although expression on the surface membrane can be upregulated by stimuli including insulin in 3T3-L1 adipocytes (Morris et al. J. Biol. Chem. (1998) 273:3582-

3587) and neurotensin in embryonic neurons (Chabry et al., J. Biol. Chem. (1993), 286:17138-17144).

Modulating neurotrophin activity: the current state of the art

Certainly, current understanding of the biological roles of neurotrophins makes the neurotrophin family an attractive target for therapeutic intervention, and some methods for modulation of neurotrophin activity are known:

1) US 6,417,159 discloses a method for enhancing the effect of a neurotrophin with analogues of p75NTR367-379.
2) US 6,300,327 describes compositions and methods for potentiation of neurotrophin activity.
3) US 6,291,247 discloses methods of screening for factors that disrupt neurotrophin conformation and reduce neurotrophin biological activity.
4) US 5,516,772 describes K-252 derivatives which enhance neurotrophin-induced activity.

Summary of invention

The present invention relates to methods for modulating the activity of one or more neurotrophins in an animal and methods for treatment of a disease or disorder in an individual by modulation of neurotrophin activity. Accordingly, in one aspect the present invention relates to a method for modulating the activity of at least one neurotrophin and/or a pro-neurotrophin in an animal comprising administering to said animal a sufficient amount of an agent capable of

(i) binding to a receptor of the Vps10p-domain receptor family
and/or
(ii) interfering with binding between a receptor of the Vps10p-domain receptor family and a neurotrophin and/or proneurotrophin
and/or
(iii)modulating the expression of a receptor of the Vps10p-domain receptor family

Methods for screening for agents capable of modulating neurotrophin activity and agents selected using these screening methods are also disclosed, as are methods for determining the effect of an agent on one or more neurotrophins in cells. The present invention also pertains to methods for modulating the transport of one or more neurotrophins.

Description of Drawings

Figure 1: Examples of Vps10p-domain receptors. Their structural composition is indicated.

Figure 2: Characterization of NGF binding to p75, TrkA, and Sortilin as measured by surface plasmon resonance analysis (BIAcore). Binding of 50-500 nM NGF was measured to 91.5 fmol/mm^2 immobilized p75-IgG-Fc chimeric protein (upper panel), to 66 fmol/mm^2 immobilized TrkA-IgG-Fc (middle panel), and to 51 fmol/mm^2 purified Sortilin extracellular domain (lower panel). The on and off rates – 100 to 600 seconds and 600 to 1000 seconds, respectively – were recorded and the Kd values for NGF binding were calculated to ~ 1 nM for p75, ~2 nM for TrkA, and ~ 87 nM for Sortilin.

Mature murine NGF was from Austral Biologicals (San Ramon, CA), recombinant human p75 neurotrophin receptor/Fc and human TrkA/Fc chimeras were from R&D systems (Oxon, UK). Human Sortilin was produced in stably transfected CHO-cells and purified as described elsewhere (Munck Petersen et al, EMBO J. (1999) 18:595-604).

Figure 3: Characterization of proNGF binding to p75, TrkA, and Sortilin as measured by surface plasmon resonance analysis (BIAcore). Binding of 25-500 nM proNGF was measured to 91.5 fmol/mm^2 immobilized p75-IgG-Fc chimeric protein (upper panel), to 66 fmol/mm^2 immobilized TrkA-IgG-Fc (middle panel), and to 51 fmol/mm^2 purified Sortilin extracellular domain (lower panel). The on and off rates – 100 to 600 seconds and 600 to 1000 seconds, respectively – were recorded and the Kd values for proNGF binding were calculated to ~ 12 nM for p75, ~15 nM for TrkA, and ~ 5 nM for Sortilin.

Human recombinant proNGF was produced and purified in E. coli as described (Rattenholl et al, Eur. J. Biochem. (2001) 268:3296-3303). All other reagents were as described in the legend to figure 2.

Figure 4: Characterization of binding of the proNGF propeptide to p75, TrkA, and Sortilin as measured by surface plasmon resonance analysis (BIAcore). Binding of 25-500 nM propeptide was measured to 91.5 fmol/mm^2 immobilized p75-IgG-Fc chimeric protein (upper panel), to 66 fmol/mm^2 immobilized TrkA-IgG-Fc (middle panel), and to 51 fmol/mm^2 purified Sortilin extracellular domain (lower panel). The on and off rates – 100 to 600 seconds and 600 to 1000 seconds, respectively – were recorded and the Kd values for proNGF propeptide binding were calculated to ~ 87 nM for Sortilin. There was no detectable binding to p75 and TrkA.

The human NGF-propeptide expressed in E.Coli was provided by Elisabeth Schwarz, Martin-Luther-Universität Halle-Wittenberg, Halle/Saale, Germany. All other reagents were as described in the legends to figures 2 and 3.

Figure 5: Inhibition of proNGF binding to immobilized Sortilin by neurotensin as measured by BIAcore analysis. Binding of 200 nM proNGF to 51 fmol/mm^2 immobilized Sortilin is inhibited by ~45% following coinjection with 10 μM neurotensin. Binding of neurotensin alone is shown for comparison.

Neurotensin was obtained from Sigma-Aldrich (St. Louis, MO). All other products were as indicated above.

Figure 6: Inhibition of proNGF binding to immobilized Sortilin by RAP (receptor-associated protein), the propeptide of proNGF, and the Sortilin propeptide. The inhibitors were prebound to Sortilin followed by coinjection with 200 nM proNGF. The baselines have been corrected for the signals obtained in the presence of each of the inhibitors. Maximal proNGF binding is measured without preincubation with the respective inhibitors. Binding of 200 nM proNGF to 51 fmol/mm^2 immobilized Sortilin is inhibited ~65% by 10 μM RAP, ~85% by 5 μM og the proNGF propeptide and ~65% by 5 μM the Sortilin propeptide.

Figure 7: Characterization of proBDNF and BDNF binding to purified Sortilin as measured by surface plasmon resonance (BIAcore). Rat proBDNF was produced in 293 cells as described in Lee, R., Kermani, P., Teng, K.K. & Hempstead, B.L. Regulation of cell survival by secreted proneurotrophins. Science 294, 1945-1948 (2001). and purified from the conditioned medium. Mature recombinant human BDNF was from Promega (#G1491) and the pro-domain of human BDNF fused to GST (glutathione S-transferase) was produced in E. coli and purified by glutathione-sepharose affinity chromatography. Binding of 5-500 nM proBDNF (upper panel), prodomain of proBDNF (a GST-fusion protein, middle panel) or BDNF (lower panel) was measured to 94 fmol/mm2 immobilized purified Sortilin extracellular domain. The experiment was carried out essentially as described for figures 2-4. The on and off rates – 100 to 600 seconds and 600 to 10000 seconds, respectively – were recorded and the Kd values for ligand binding were calculated to ~3 nM for proBDNF, ~58 nM for the GST-pro-domain of proBDNF, and ~40 nM for mature BDNF.

Other preparations of mature BDNF have shown Kd values for ligand binding at 10 nM.

Description of Sequences

SEQ ID NO 1: Sortilin sequence
SEQ ID NO 2: SorLA sequence
SEQ ID NO 3: SorCS1 sequence
SEQ ID NO 4: SorCS3 sequence
SEQ ID NO 5: SorCS2 sequence
SEQ ID NO 6: NGF sequence
SEQ ID NO 7: BDNF sequence
SEQ ID NO 8: neurotrophin-3 sequence
SEQ ID NO 9: neurotrophin-4 sequence
SEQ ID NO 10: neurotensin sequence
SEQ ID NO 11: neuromedin sequence
SEQ ID NO 12: Receptor associated peptide (RAP)
SEQ ID NO 13: pro-neurotensin/pro-neuromedin

Detailed description of the invention

Definitions

The term "binding" as used herein refers to the transient or longer lasting attraction or binding of two or more moieties to one another, mediated by physical forces such as e.g. electrostatic interactions, hydrophobic interactions, dipole-dipole interactions and hydrogen bonds. The term "hydrophobic interaction" as used herein refers to any interaction occurring between essentially non-polar (hydrophobic) components located within attraction range of one another in a polar environment (e.g. water). As used herein, attraction range is on the scale of about 100 nm. A particular type of hydrophobic interaction is exerted by "Van der Waal's forces", i.e. the attractive forces between non-polar molecules that are accounted for by quantum mechanics. Van der Waal's forces are generally associated with momentary dipole moments which are induced by neighbouring molecules and which involve changes in electron distribution. The term "hydrogen bond" as used herein refers to an attractive force, or bridge, which may occur between a hydrogen atom which is bonded covalently to an electronegative atom, for example, oxygen, sulphur, or nitrogen, and another electronegative atom. The hydrogen bond may occur between a hydrogen atom in a first molecule and an electronegative atom in a second molecule (intermolecular hydrogen bonding). Also, the hydrogen bond may occur between a hydrogen atom and an electronegative atom which are both contained in a single molecule (intramolecular hydrogen bonding). The term "electrostatic interaction" as used herein refers to any interaction occurring between charged components, molecules or ions, due to attractive forces when components of opposite electric charge are attracted to each other. Examples include, but are not limited to: ionic interactions, covalent interactions, interactions between a ion and a dipole (ion and polar molecule), interactions between two dipoles (partial charges of polar molecules), hydrogen bonds and London dispersion bonds (induced dipoles of polarizable molecules). Thus, for example, "ionic interaction" or "electrostatic interaction" refers to the attraction between a first, positively charged molecule and a second, negatively charged molecule. Ionic or electrostatic interactions include, for example, the attraction between a negatively charged bioactive agent (input examples relevant to this invention). The term "dipole-dipole interaction" as used herein refers to the attraction which can occur among two or more polar molecules. Thus, "dipole-dipole interaction" refers to

the attraction of the uncharged, partial positive end of a first polar molecule to the uncharged, partial negative end of a second polar molecule. "Dipole-dipole interaction" also refers to intermolecular hydrogen bonding.

Functional equivalents and variants of polynucleotides encoding a neurotrophin activity modulator and polypeptides comprising such a neurotrophin activity modulator: "functional equivalents" and "variants" are used interchangeably herein. In one preferred embodiment of the invention there is also provided variants of neurotrophin activity modulator and variants of fragments thereof. When being polypeptides, variants are determined on the basis of their degree of identity or their homology with a predetermined amino acid sequence, said predetermined amino acid sequence being one of SEQ ID NO: neurotrophin activity modulator, or, when the variant is a fragment, a fragment of any of the aforementioned amino acid sequences, respectively.

Accordingly, variants preferably have at least 75% sequence identity, for example at least 80% sequence identity, such as at least 85% sequence identity, for example at least 90% sequence identity, such as at least 91% sequence identity, for example at least 91% sequence identity, such as at least 92% sequence identity, for example at least 93% sequence identity, such as at least 94% sequence identity, for example at least 95% sequence identity, such as at least 96% sequence identity, for example at least 97% sequence identity, such as at least 98% sequence identity, for example 99% sequence identity with the predetermined sequence.

Sequence identity is determined in one embodiment by utilising fragments of neurotrophin activity modulator peptides comprising at least 25 contiguous amino acids and having an amino acid sequence which is at least 80%, such as 85%, for example 90%, such as 95%, for example 99% identical to the amino acid sequence of any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, and SEQ ID NO: 8, respectively, wherein the percent identity is determined with the algorithm GAP, BESTFIT, or FASTA in the Wisconsin Genetics Software Package Release 7.0, using default gap weights.

The following terms are used to describe the sequence relationships between two or more polynucleotides: "predetermined sequence", "comparison window", "sequence identity", "percentage of sequence identity", and "substantial identity".

A "predetermined sequence" is a defined sequence used as a basis for a sequence comparison; a predetermined sequence may be a subset of a larger sequence, for example, as a segment of a full-length DNA or gene sequence given in a sequence listing, such as a polynucleotide sequence of SEQ ID NO:1, or may comprise a complete DNA or gene sequence. Generally, a predetermined sequence is at least 20 nucleotides in length, frequently at least 25 nucleotides in length, and often at least 50 nucleotides in length.

Since two polynucleotides may each (1) comprise a sequence (i.e., a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) may further comprise a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window", as used herein, refers to a conceptual segment of at least 20 contiguous nucleotide positions wherein a polynucleotide sequence may be compared to a predetermined sequence of at least 20 contiguous nucleotides and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) of 20 percent or less as compared to the predetermined sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences.

Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2: 482, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48: 443, by the search for similarity method of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. (U.S.A.) 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by inspection, and the best alignment (i.e., resulting in the

highest percentage of homology over the comparison window) generated by the various methods is selected.

The term "sequence identity" means that two polynucleotide sequences are identical (i.e., on a nucleotide-by-nucleotide basis) over the window of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, U, or I) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The terms "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 85 percent sequence identity, preferably at least 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a predetermined sequence over a comparison window of at least 20 nucleotide positions, frequently over a window of at least 25-50 nucleotides, wherein the percentage of sequence identity is calculated by comparing the predetermined sequence to the polynucleotide sequence which may include deletions or additions which total 20 percent or less of the predetermined sequence over the window of comparison. The predetermined sequence may be a subset of a larger sequence, for example, as a segment of the full-length SEQ ID NO:1 polynucleotide sequence illustrated herein.

As applied to polypeptides, a degree of identity of amino acid sequences is a function of the number of identical amino acids at positions shared by the amino acid sequences. A degree of homology or similarity of amino acid sequences is a function of the number of amino acids, i.e. structurally related, at positions shared by the amino acid sequences.

An "unrelated" or "non-homologous" sequence shares less than 40% identity, though preferably less than 25% identity, with one of the neurotrophin activity modulator polypeptide sequences of the present invention. The term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, preferably at least 90 percent sequence identity, more preferably at

least 95 percent sequence identity or more (e.g., 99 percent sequence identity). Preferably, residue positions which are not identical differ by conservative amino acid substitutions.

Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine, a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulphur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine.

Additionally, variants are also determined based on a predetermined number of conservative amino acid substitutions as defined herein below. Conservative amino acid substitution as used herein relates to the substitution of one amino acid (within a predetermined group of amino acids) for another amino acid (within the same group), wherein the amino acids exhibit similar or substantially similar characteristics.

Within the meaning of the term "conservative amino acid substitution" as applied herein, one amino acid may be substituted for another within the groups of amino acids indicated herein below:

i) Amino acids having polar side chains (Asp, Glu, Lys, Arg, His, Asn, Gln, Ser, Thr, Tyr, and Cys,)

ii) Amino acids having non-polar side chains (Gly, Ala, Val, Leu, Ile, Phe, Trp, Pro, and Met)

iii) Amino acids having aliphatic side chains (Gly, Ala Val, Leu, Ile)

iv) Amino acids having cyclic side chains (Phe, Tyr, Trp, His, Pro)

v) Amino acids having aromatic side chains (Phe, Tyr, Trp)

vi) Amino acids having acidic side chains (Asp, Glu)

vii) Amino acids having basic side chains (Lys, Arg, His)

viii) Amino acids having amide side chains (Asn, Gln)

ix) Amino acids having hydroxy side chains (Ser, Thr)

x) Amino acids having sulphur-containing side chains (Cys, Met),

xi) Neutral, weakly hydrophobic amino acids (Pro, Ala, Gly, Ser, Thr)

xii) Hydrophilic, acidic amino acids (Gln, Asn, Glu, Asp), and

xiii) Hydrophobic amino acids (Leu, Ile, Val)

Accordingly, a variant or a fragment thereof according to the invention may comprise, within the same variant of the sequence or fragments thereof, or among different variants of the sequence or fragments thereof, at least one substitution, such as a plurality of substitutions introduced independently of one another.

It is clear from the above outline that the same variant or fragment thereof may comprise more than one conservative amino acid substitution from more than one group of conservative amino acids as defined herein above.

The addition or deletion of at least one amino acid may be an addition or deletion of from preferably 2 to 250 amino acids, such as from 10 to 20 amino acids, for example from 20 to 30 amino acids, such as from 40 to 50 amino acids. However, additions or deletions of more than 50 amino acids, such as additions from 50 to 100 amino acids, addition of 100 to 150 amino acids, addition of 150-250 amino acids,

are also comprised within the present invention. The deletion and/or the addition may - independently of one another - be a deletion and/or an addition within a sequence and/or at the end of a sequence.

The polypeptide fragments according to the present invention, including any functional equivalents thereof, may in one embodiment comprise less than 250 amino acid residues, such as less than 240 amino acid residues, for example less than 225 amino acid residues, such as less than 200 amino acid residues, for example less than 180 amino acid residues, such as less than 160 amino acid residues, for example less than 150 amino acid residues, such as less than 140 amino acid residues, for example less than 130 amino acid residues, such as less than 120 amino acid residues, for example less than 110 amino acid residues, such as less than 100 amino acid residues, for example less than 90 amino acid residues, such as less than 85 amino acid residues, for example less than 80 amino acid residues, such as less than 75 amino acid residues, for example less than 70 amino acid residues, such as less than 65 amino acid residues, for example less than 60 amino acid residues, such as less than 55 amino acid residues, for example less than 50 amino acid residues.

"Functional equivalency" as used in the present invention is, according to one preferred embodiment, established by means of reference to the corresponding functionality of a predetermined fragment of the sequence.

Functional equivalents or variants of a neurotrophin activity modulator will be understood to exhibit amino acid sequences gradually differing from the preferred predetermined neurotrophin activity modulator sequence, as the number and scope of insertions, deletions and substitutions including conservative substitutions increase. This difference is measured as a reduction in homology between the preferred predetermined sequence and the fragment or functional equivalent.

All fragments or functional equivalents of SEQ ID NO: neurotrophin activity modulator are included within the scope of this invention, regardless of the degree of homology that they show to the respective, predetermined neurotrophin activity modulator sequences disclosed herein. The reason for this is that some regions of the neurotrophin activity modulator are most likely readily mutatable, or capable of

being completely deleted, without any significant effect on the binding activity of the resulting fragment.

A functional variant obtained by substitution may well exhibit some form or degree of native neurotrophin activity modulator activity, and yet be less homologous, if residues containing functionally similar amino acid side chains are substituted. Functionally similar in this respect refers to dominant characteristics of the side chains such as hydrophobic, basic, neutral or acidic, or the presence or absence of steric bulk. Accordingly, in one embodiment of the invention, the degree of identity is not a principal measure of a fragment being a variant or functional equivalent of a preferred predetermined fragment according to the present invention.

The homology between amino acid sequences may be calculated using well known scoring matrices such as any one of BLOSUM 30, BLOSUM 40, BLOSUM 45, BLOSUM 50, BLOSUM 55, BLOSUM 60, BLOSUM 62, BLOSUM 65, BLOSUM 70, BLOSUM 75, BLOSUM 80, BLOSUM 85, and BLOSUM 90.

Fragments sharing homology with fragments of SEQ ID NO:1-13, respectively, are to be considered as falling within the scope of the present invention when they are preferably at least about 90 percent homologous, for example at least 92 percent homologous, such as at least 94 percent homologous, for example at least 95 percent homologous, such as at least 96 percent homologous, for example at least 97 percent homologous, such as at least 98 percent homologous, for example at least 99 percent homologous with said predetermined fragment sequences, respectively. According to one embodiment of the invention, the homology percentages refer to identity percentages.

Additional factors that may be taken into consideration when determining functional equivalence according to the meaning used herein are i) the ability of antisera to detect a neurotrophin activity modulator fragment according to the present invention, or ii) the ability of the functionally equivalent neurotrophin activity modulator fragment to compete with the corresponding neurotrophin activity modulator in an assay. One method of determining a sequence of immunogenically active amino acids within a known amino acid sequence has been described by Geysen in US 5,595,915 and is incorporated herein by reference.

A further suitably adaptable method for determining structure and function relationships of peptide fragments is described in US 6,013,478, which is herein incorporated by reference. Also, methods of assaying the binding of an amino acid sequence to a receptor moiety are known to the skilled artisan.

In addition to conservative substitutions introduced into any position of a preferred predetermined neurotrophin activity modulator, or a fragment thereof, it may also be desirable to introduce non-conservative substitutions in any one or more positions of such a neurotrophin activity modulator.

A non-conservative substitution leading to the formation of a functionally equivalent fragment of neurotrophin activity modulator would for example i) differ substantially in polarity, for example a residue with a non-polar side chain (Ala, Leu, Pro, Trp, Val, Ile, Leu, Phe or Met) substituted for a residue with a polar side chain such as Gly, Ser, Thr, Cys, Tyr, Asn, or Gln or a charged amino acid such as Asp, Glu, Arg, or Lys, or substituting a charged or a polar residue for a non-polar one; and/or ii) differ substantially in its effect on polypeptide backbone orientation such as substitution of or for Pro or Gly by another residue; and/or iii) differ substantially in electric charge, for example substitution of a negatively charged residue such as Glu or Asp for a positively charged residue such as Lys, His or Arg (and vice versa); and/or iv) differ substantially in steric bulk, for example substitution of a bulky residue such as His, Trp, Phe or Tyr for one having a minor side chain, e.g. Ala, Gly or Ser (and vice versa).

Variants obtained by substitution of amino acids may in one preferred embodiment be made based upon the hydrophobicity and hydrophilicity values and the relative similarity of the amino acid side-chain substituents, including charge, size, and the like. Exemplary amino acid substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

In addition to the variants described herein, sterically similar variants may be formulated to mimic the key portions of the variant structure and that such

compounds may also be used in the same manner as the variants of the invention. This may be achieved by techniques of modelling and chemical designing known to those of skill in the art. It will be understood that all such sterically similar constructs fall within the scope of the present invention.

In a further embodiment the present invention relates to functional variants comprising substituted amino acids having hydrophilic values or hydropathic indices that are within +/-4.9, for example within +/-4.7, such as within +/-4.5, for example within +/-4.3, such as within +/-4.1, for example within +/-3.9, such as within +/-3.7, for example within +/- 3.5, such as within +/-3.3, for example within +/- 3.1, such as within +/- 2.9, for example within +/- 2.7, such as within +/-2.5, for example within +/- 2.3, such as within +/- 2.1, for example within +/- 2.0, such as within +/- 1.8, for example within +/- 1.6, such as within +/- 1.5, for example within +/- 1.4, such as within +/- 1.3 for example within +/- 1.2, such as within +/- 1.1, for example within +/- 1.0, such as within +/- 0.9, for example within +/- 0.8, such as within +/- 0.7, for example within +/- 0.6, such as within +/- 0.5, for example within +/- 0.4, such as within +/- 0.3, for example within +/- 0.25, such as within +/- 0.2 of the value of the amino acid it has substituted.

The importance of the hydrophilic and hydropathic amino acid indices in conferring interactive biologic function on a protein is well understood in the art (Kyte & Doolittle, 1982 and Hopp, U.S. Pat. No. 4,554,101, each incorporated herein by reference).

The amino acid hydropathic index values as used herein are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5) (Kyte & Doolittle, 1982).

The amino acid hydrophilicity values are: arginine (+3.0); lysine (+3.0); aspartate (+3.0.+-.1); glutamate (+3.0.+-.1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5.+-.1); alanine (-0.5); histidine (-0.5);

cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4) (U.S. 4,554,101).

In addition to the peptidyl compounds described herein, sterically similar compounds may be formulated to mimic the key portions of the peptide structure and that such compounds may also be used in the same manner as the peptides of the invention. This may be achieved by techniques of modelling and chemical designing known to those of skill in the art. For example, esterification and other alkylations may be employed to modify the amino terminus of, e.g., a di-arginine peptide backbone, to mimic a tetra peptide structure. It will be understood that all such sterically similar constructs fall within the scope of the present invention.

Peptides with N-terminal alkylations and C-terminal esterifications are also encompassed within the present invention. Functional equivalents also comprise glycosylated and covalent or aggregative conjugates formed with the same or other neurotrophin activity modulator fragments and/or neurotrophin activity modulator molecules, including dimers or unrelated chemical moieties. Such functional equivalents are prepared by linkage of functionalities to groups which are found in fragment including at any one or both of the N- and C-termini, by means known in the art.

Functional equivalents may thus comprise fragments conjugated to aliphatic or acyl esters or amides of the carboxyl terminus, alkylamines or residues containing carboxyl side chains, e.g., conjugates to alkylamines at aspartic acid residues; O-acyl derivatives of hydroxyl group-containing residues and N-acyl derivatives of the amino terminal amino acid or amino-group containing residues, e.g. conjugates with fMet-Leu-Phe or immunogenic proteins. Derivatives of the acyl groups are selected from the group of alkyl-moieties (including C3 to C10 normal alkyl), thereby forming alkanoyl species, and carbocyclic or heterocyclic compounds, thereby forming aroyl species. The reactive groups preferably are difunctional compounds known per se for use in cross-linking proteins to insoluble matrices through reactive side groups.

Covalent or aggregative functional equivalents and derivatives thereof are useful as reagents in immunoassays or for affinity purification procedures. For example, a fragment of neurotrophin activity modulator according to the present invention may be insolubilized by covalent bonding to cyanogen bromide-activated Sepharose by

methods known per se or adsorbed to polyolefin surfaces, either with or without glutaraldehyde cross-linking, for use in an assay or purification of anti-neurotrophin activity modulator antibodies or cell surface receptors. Fragments may also be labelled with a detectable group, e.g., radioiodinated by the chloramine T procedure, covalently bound to rare earth chelates or conjugated to another fluorescent moiety for use in e.g. diagnostic assays.

Mutagenesis of a preferred predetermined fragment of neurotrophin activity modulator can be conducted by making amino acid insertions, usually on the order of about from 1 to 10 amino acid residues, preferably from about 1 to 5 amino acid residues, or deletions of from about from 1 to 10 residues, such as from about 2 to 5 residues.

In one embodiment the fragment of neurotrophin activity modulator is synthesised by automated synthesis. Any of the commercially available solid-phase techniques may be employed, such as the Merrifield solid phase synthesis method, in which amino acids are sequentially added to a growing amino acid chain (see Merrifield, J. Am. Chem. Soc. 85:2149-2146, 1963).

Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Applied Biosystems, Inc. of Foster City, Calif., and may generally be operated according to the manufacturer's instructions. Solid phase synthesis will enable the incorporation of desirable amino acid substitutions into any fragment of neurotrophin activity modulator according to the present invention. It will be understood that substitutions, deletions, insertions or any subcombination thereof may be combined to arrive at a final sequence of a functional equivalent. Insertions shall be understood to include amino-terminal and/or carboxyl-terminal fusions, e.g. with a hydrophobic or immunogenic protein or a carrier such as any polypeptide or scaffold structure capable as serving as a carrier.

Oligomers including dimers including homodimers and heterodimers of fragments of neurotrophin activity modulator according to the invention are also provided and fall under the scope of the invention. Neurotrophin activity modulator functional equivalents and variants can be produced as homodimers or heterodimers with other amino acid sequences or with native neurotrophin activity modulator sequences.

Heterodimers include dimers containing immunoreactive neurotrophin activity modulator fragments as well as neurotrophin activity modulator fragments that need not have or exert any biological activity.

Neurotrophin activity modulator fragments according to the invention may be synthesised both in vitro and in vivo. Method for in vitro synthesis are well known, and methods being suitable or suitably adaptable to the synthesis in vivo of neurotrophin activity modulator are also described in the prior art. When synthesized in vivo, a host cell is transformed with vectors containing DNA encoding neurotrophin activity modulator or a fragment thereof. A vector is defined as a replicable nucleic acid construct. Vectors are used to mediate expression of neurotrophin activity modulator. An expression vector is a replicable DNA construct in which a nucleic acid sequence encoding the predetermined neurotrophin activity modulator fragment, or any functional equivalent thereof that can be expressed in vivo, is operably linked to suitable control sequences capable of effecting the expression of the fragment or equivalent in a suitable host. Such control sequences are well known in the art.

Cultures of cells derived from multicellular organisms represent preferred host cells. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of useful host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, and WI38, BHK, COS-7, 293 and MDCK cell lines. Preferred host cells are eukaryotic cells known to synthesize endogenous neurotrophin activity modulator. Cultures of such host cells may be isolated and used as a source of the fragment, or used in therapeutic methods of treatment, including therapeutic methods aimed at promoting or inhibiting a growth state, or diagnostic methods carried out on the human or animal body.

Pharmaceutical agent: the terms "pharmaceutical agent" or "drug" or "medicament" refer to any therapeutic or prophylactic agent which may be used in the treatment (including the prevention, diagnosis, alleviation, or cure) of a malady, affliction, condition, disease or injury in a patient. Therapeutically useful genetic determinants, peptides, polypeptides and polynucleotides may be included within the meaning of the term pharmaceutical or drug. As defined herein, a "therapeutic agent," "pharmaceutical agent" or "drug" or "medicament" is a type of bioactive agent.

The term “bioactive agent” as used herein refers to any a substance which may be used in connection with an application that is therapeutic or diagnostic, such as, for example, in methods for diagnosing the presence or absence of a disease in a patient and/or methods for the treatment of a disease in a patient. "Bioactive agent" refers to substances, which are capable of exerting a biological effect in vitro and/or in vivo. The bioactive agents may be neutral, positively or negatively charged. Suitable bioactive agents include, for example, prodrugs, diagnostic agents, therapeutic agents, pharmaceutical agents, drugs, oxygen delivery agents, blood substitutes, synthetic organic molecules, polypeptides, peptides, vitamins, steroids, steroid analogues and genetic determinants, including nucleosides, nucleotides and polynucleotides.

Treatment: the term “treatment” as used herein refers to a method involving therapy including surgery of a clinical condition in an individual including a human or animal body. The therapy may be prophylactic, ameliorating or curative.

antisense-RNA: an RNA molecule capable of causing gene silencing by specifically binding to an mRNA molecule of interest.
antisense-DNA: a DNA molecule capable of causing gene silencing by specifically binding to an mRNA molecule of interest.
SiRNA: “small interfering RNA” (SiRNA) is a short (often, but not restricted to, less than 30 nucleotides long) double-stranded RNA molecule capable of causing gene-specific silencing in mammalian cells

Gene "silencing": a process leading to reduced expression of endogenous genes. Gene silencing is preferably the result of post-transcriptional reduction of gene expression.

Up-regulation of expression: a process leading to increased expression of genes, preferably of endogenous genes.

In vitro/in vivo: the terms are used in their normal meaning.

Polypeptide: The term "polypeptide" as used herein refers to a molecule comprising at least two amino acids. The amino acids may be natural or synthetic. "Oligopeptides" are defined herein as being polypeptides of length not more than 100 amino acids. The term "polypeptide" is also intended to include proteins, i.e. functional biomolecules comprising at least one polypeptide; when comprising at least two polypeptides, these may form complexes, be covalently linked or may be non-covalently linked. The polypeptides in a protein can be glycosylated and/or lipidated and/or comprise prosthetic groups.

"Polynucleotide" as used herein refers to a molecule comprising at least two nucleic acids. The nucleic acids may be naturally occurring or modified, such as locked nucleic acids (LNA), or peptide nucleic acids (PNA). Polynucleotide as used herein generally pertains to

i) a polynucleotide comprising a predetermined coding sequence, or

ii) a polynucleotide encoding a predetermined amino acid sequence, or

iii) a polynucleotide encoding a fragment of a polypeptide encoded by polynucleotides (i) or (ii), wherein said fragment has at least one predetermined activity as specified herein; and

iv) a polynucleotide the complementary strand of which hybridizes under stringent conditions with a polynucleotide as defined in any one of (i), (ii) and (iii), and encodes a polypeptide, or a fragment thereof, having at least one predetermined activity as specified herein; and

v) a polynucleotide comprising a nucleotide sequence which is degenerate to the nucleotide sequence of polynucleotides (iii) or (iv);

or the complementary strand of such a polynucleotide.

A "purified antibody" is an antibody at least 60 weight percent of which is free from the polypeptides and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation comprises antibody in an amount of at least

75 weight percent, more preferably at least 90 weight percent, and most preferably at least 99 weight percent.

Detailed description

The present inventors have identified that neurotrophins bind to receptors of the Vps10p-domain receptor family. Accordingly, the present invention relates to modulation of the activity of at least one neurotrophin.

Without being bound by theory it is believed that Vps10p-domain receptor family is involved in one or more of the following mechanisms in relation to neurotrophins:

- Retrograde transport, including uptake of proneurotrophin, neurotrophin and p75

- Transport within biosynthetic pathways, including sorting of proneurotrophin and transport from the Golgi network

- Release of neurotrophins

- Signalling, including modulation of cellular transport and signalling by formation of ternary complexes with p75 and neurotrophin or pro-neurotrophin

Thus, one aspect of the present invention is a method for modulating the activity of at least one neurotrophin and/or a pro-neurotrophin in a single cell or an organism, including an animal, comprising administering to said animal a sufficient amount of an agent capable of binding to a receptor of the Vps10p-domain receptor family or capable of interfering with binding between a receptor of the Vps10p-domain receptor family and a neurotrophin and/or proneurotrophin.

Receptors of the Vps10p-domain receptor family

The term "receptor of the Vps10p family" refers to a family of receptors characterised by having an N-terminal Vps10p domain; said Vpsp10p domain family comprises SorLA, Sortilin, SorCS1, SorCS-2, or SorCS-3, see Fig. 1. In one embodiment of the present invention, any of the receptors of the Vps10p domain family may be used; more preferably, the receptor comprises the Vps10p domain,

the 10CC module, a transmembrane segment as well as a cytoplasmic segment mediating cellular sorting and internalization as well as mediating binding to cytoplasmic adaptors affecting cellular signalling. In particular the receptor used is Sortilin.

Neurotrophins/pro-neurotrophins

The term "neurotrophin" as used herein refers to any member of the neurotrophin family, said neurotrophin family comprising neural growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5). In one embodiment of the present invention, any member of the neurotrophin family may be used; however, it is preferred that the neurotrophin is NGF or BDNF.

The term "pro-neurotrophin" as used herein may refer to any pro-peptide of the neurotrophin family, said family of pro-peptides comprising pro-NGF, pro-BDNF, pro-NT-3 and pro-NT-4/5. In one embodiment of the present invention, any pro-neurotrophin may be used, however it is preferred that the pro-neurotrophin is pro-NGF or pro-BDNF.

Modulation of neurotrophin activity

The terms "neurotrophin-mediated" activity, "activity of a neurotrophin" or "neurotrophin activity" refer to a biological activity that is normally promoted, either directly or indirectly, in the presence of a neurotrophin or a proneurotrophin. Neurotrophin activities include, but are not restricted to, neuronal survival, neuronal differentiation including process formation and neurite outgrowth, biochemical changes such as enzyme induction, involvement in depression and antidepressant action, involvement in accelerating nerve process growth, and involvement in decreasing general cell motility. It has been hypothesized that the lack of neurotrophic factors is responsible for the degeneration of selective neuronal populations as it occurs in Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis.

The activities of pro-neurotrophins include, but are not restricted to, differentially activating both pro- and anti-apoptotic cellular responses, through preferential activation of p75 ^{NTR} or TrkA receptors respectively.

In preferred embodiments of the present invention, one ore more of these activities of neurotrophin(s) and/or proneurotrophin(s) are modulated directly or indirectly by the administration of an agent to an animal.

The terms "modulation" or "modulated" refer to any change or changes in the biological activity of a bioactive agent, for example a neurotrophin. In one embodiment of the present invention, such a modulation of activity of a neurotrophin is a decrease in the neurotrophin activity; however, the modulation may equally be an increase in neurotrophin activity.

Agents capable of modulating activity

In one preferred embodiment of the present invention, an agent is administered to the animal, said agent being capable of modulating the binding between a receptor of the Vps10p-domain receptor family and a neurotrophin and/or proneurotrophin.

In another, equally preferred embodiment, the agent is capable of binding to a receptor of the Vps10p-domain receptor family or a neurotrophin and/or pro-neurotrophin thereby interfering with the activity of a neurotrophin, either directly or indirectly.

In a third, equally preferred embodiment of the present invention, the agent is capable of modulating the expression of a receptor of the Vps10p-domain receptor family.

The agent capable of exhibiting one or more of the above mentioned effects may be any type of agent, for example the agent may be selected from the group comprising proteins, peptides, polypeptides, antibodies, antisense-RNA, antisense-DNA, siRNA, other polynucleotides, or organic molecules, In a preferred embodiment the agent is an antibody or a polypeptide, and the agent is most preferably a polypeptide.

In a particularly preferred embodiment of the present invention, the agent administered to the animal is capable of modulating the activity of a sortilin receptor in relation to a neurotrophin, said activity may be, but is not restricted to, one or more of the following:

i) cellular sorting of the receptor
ii) receptor binding directly or indirectly by ligand bridging to other receptors, such as the p75 and Trk receptors
iii) sortilin receptor signalling

In one embodiment of the present invention, the agent is capable of inhibiting binding of a neurotrophin or pro-neurotrophin to a receptor of the Vps10p-domain receptor family. Such inhibition may for example be due to binding of the agent either to the neurotrophin and/or the pro-neurotrophin and/or the receptor.

In one embodiment the agent is capable of binding the neurotrophin and/or proneurotrophin, such as a soluble receptor of the Vps10p-domain receptor family or a fragment or a variant thereof, said fragment or variant being capable of binding said neurotrophin. In particular the soluble receptor is a soluble Sortilin receptor, or a fragment or a variant thereof. Any fragment or variant capable of binding to a neurotrophin and/or a pro-neurotrophin is included herein. In particular a fragment is a peptide comprising a sequence corresponding to the 10CC motif of the Vps10p-domain receptor family having the sequence of SEQ ID NO: 1 amino acid residue 612-740 or a fragment or variant thereof.

In another embodiment the agent is capable of binding to the receptor. The agent may bind to any part of the receptor relevant for inhibiting the binding of the neurotrophin. Accordingly, the agent may be capable of inhibiting the binding of said neurotrophin or said pro-neurotrophin to a receptor of the Vps10p-domain receptor family by binding to an extracellular part of the receptor, an intracellular part of the receptor, or a segment of the transmembrane part of the receptor.

An example of an agent according to the invention is an antibody directed against an extra-cellular part of the receptor. In an even more preferred embodiment, the antibody is purified. In the preferred embodiment wherein the agent is an antibody

directed against an extra-cellular part of the receptor, the antibody is preferably directed against a peptide comprising a sequence corresponding to the 10CC motif of the Vps10p-domain receptor family having the sequence of SEQ ID NO: 1 amino acid residue 612-740 or a fragment or variant thereof. In particular the antibody should be directed against a position in this motif so that the antibody sterically blocks the binding of the neurotrophin and/or pro-neurotrophin to the receptor.

In yet another embodiment, the agent is a peptide comprising a sequence having SEQ ID NO: 1 amino acid residue 34-77 corresponding of the part of the pro-Sortilin sequence binding Sortilin or a fragment or variant thereof, said peptide being capable of binding to the receptor. The fragment thereof preferably comprises SEQ ID NO: 1 amino acid residue 50-70, more preferably SEQ ID NO: 1 amino acid residue 55-61 (GVSWGLR).

In another preferred embodiment, the agent is selected from one or more of the following sequences: SEQ ID NO: 2 amino acid residue 29-81 corresponding to the propart from SorLa, or a fragment or a variant thereof. In particular a fragment or a variant thereof should comprise a sequence corresponding to the sequence SEQ ID NO: 2 amino acid residues 47-66.

In yet another preferred embodiment of the present invention, the agent is a peptide which comprises one or more of the following sequences or a fragment or variant thereof: SEQ ID NO: 6 amino acid residue 19-121 (propart for NGF), SEQ ID NO: 7 amino acid residue 19-127 (propart for BDNF), SEQ ID NO: 8 amino acid residue 17-124 (propart for neurotrophin-3 (NT-3), SEQ ID NO: 9 amino acid residue 25-80 (propart for neurotrophin-4 (NT-4), or a fragment or a variant thereof, said peptide being capable of binding to the receptor. The agent is even more preferably a peptide comprising a Sortilin receptor-binding sequence of proNGF or a fragment or variant thereof. The agent in another preferred embodiment may be a peptide comprising the sequence SEQ ID NO: 6 amino acid residue 19-121 (the sequence from the pro-part of NGF) or a fragment or variant thereof, said peptide being capable of binding to the receptor.

In another preferred embodiment of the present invention, the agent may preferably be a peptide having the sequence of SEQ ID NO: 13, the sequence for the pro-

neurotensin/pro-neuromedin, SEQ ID NO: 10 (the sequence of neurotensin), SEQ ID NO: 11 (the sequence of neuromedin) or a fragment or a variant thereof, said peptide being capable of binding the receptor.

In yet another preferred embodiment of the present invention the agent may be a peptide comprising an NGF variant or a Sortilin-receptor binding fragment thereof. More preferably, this peptide comprising an NGF variant or a Sortilin-receptor binding fragment thereof is capable of binding Sortilin and stimulating the activity of the Sortilin receptor. Even more preferably, this peptide comprising an NGF variant or a Sortilin-receptor binding fragment thereof comprises one or more of the sequences disclosed in US patent No. 6,333,310 or a fragment or variant thereof (sequences for NGF variants).

In yet another embodiment the agent is derived from naturally occurring RAP (receptor-associated protein), such as a fragment or a variant of RAP. RAP (receptor-associated protein) is a cellular protein comprising about 300 amino acids, in a preferred embodiment having the sequence shown in: XM_003315, Gene: AH006949 corresponding to SEQ ID NO: 12. In a preferred embodiment the RAP derived agent is a peptide comprising a minimal functional domain having at most 104 amino acids, preferably from 20 to 60 amino acids. In particular, they are minimal functional protein domains. These peptides have at the most 104 amino acids, preferably from 20 to 60 amino acids. A preferred domain is amino acid positions 219-323 of RAP.

In another preferred embodiment of the present invention, the agent is capable of binding to an intracellular part of the receptor and/or the transmembrane part of a receptor of the Vsp10p domain receptor family. In particular the agent may be capable of binding to the cytoplasmic part of the receptor of the Vsp10p domain receptor family, such as to a part of Sortilin corresponding to SEQ ID NO: 1 amino acid residues 779-831 or a fragment of a variant thereof. More preferably the agent is capable of binding to cytoplasmic part of the receptor of the Vsp10p domain receptor family, such as to a part of Sortilin corresponding to SEQ ID NO: 1 amino acid residues 792-794 (YSVL) or amino acid residues 821-831 (HDDSDEDLLE) or a fragment of a variant thereof.

In particular binding of an agent to the intracellular and transmembrane parts of the receptor may lead to modulation of the neurotrophin and/or proneurotrophin activity through a modulation of the transport of at least one neurotrophin and/or proneurotrophin out of, into or within cells expressing the receptor of the Vsp10p domain receptor family as discussed below.

In another preferred embodiment, the agent is capable of modulating the expression of a receptor of the Vps10p-domain receptor family and thereby interfering with the activity of at least one neurotrophin. The modulation may be either inhibition or stimulation of the expression. Preferable methods for modulating the expression of the receptor include, but are not restricted to:

(i) Blocking or inhibiting the activity of the translation products of one or more Vps10p-domain receptor genes and/or one or more derivatives thereof, by inhibiting mRNA translation or transcriptional activation using antisense nucleic acids.

(ii) Inactivating mRNA by ribozymes targeted to the mRNAs encoding one or more Vps10p-domain receptor genes and/or one or more derivatives thereof.

(iii) Inhibition of the intracellularly present translation products of the Vps10p-domain receptor genes by administering molecules which mimic targets of the translation products of one or more Vps10p-domain receptor genes and/or one or more derivatives thereof thereby competing with their natural targets.

(iv) Stimulating the expression of one or more Vps10p-domain receptor genes and/or one or more derivatives thereof, for example in one preferred embodiment, an agent is administered to cells in vitro or in vivo. Such an agent may act either specifically or non-specifically. It is also possible to activate genes responsible for further growth of differentiated tissue by introducing one or more Vps10p-domain receptor genes and/or one or more derivatives thereof into the respective cells and tissue by means of gene therapy. For this purpose the respective nucleic acid sequences may be put under control of a strong promoter, which optionally can be activated and deactivated upon administration of a stimulus to the cell/tissue.

(v) Stimulating expression of one or more Vps10p-domain receptor genes and/or one or more derivatives thereof by administering directly to the re-

spective cell/tissue a translation product, either a peptide or a protein, that is derived from one or more Vps10p-domain receptor gene and/or one or more derivative thereof. Due to the low molecular weight of any of the aforementioned translation products these peptides/proteins can easily be applied to the cell, for example using encapsulation delivery systems.

The change in expression level of the receptor of the Vps10p-domain receptor family may be assayed for using methods known to those skilled in the art, including but not restricted to: DNA arrays or microarrays (Brazma and Vilo, FEBS Lett., 2000, 480, 17-24; Celis, et al., FEBS Lett., 2000, 480, 2-16), SAGE (serial analysis of gene expression)(Madden, et al., Drug Discov. Today, 2000, 5, 415-425), READS (restriction enzyme amplification of digested cDNAs) (Prashar and Weissman, Methods Enzymol., 1999, 303, 258-72), TOGA (total gene expression analysis) (Sutcliffe, et al., Proc. Natl. Acad. Sci. U. S. A., 2000, 97, 1976-81), protein arrays and proteomics (Celis, et al., FEBS Lett., 2000, 480, 2-16; Jungblut, et al., Electrophoresis, 1999, 20, 2100-10), expressed sequence tag (EST) sequencing (Celis, et al., FEBS Lett., 2000, 480, 2-16; Larsson, et al., J. Biotechnol., 2000, 80, 143-57), subtractive RNA fingerprinting (SuRF) (Fuchs, et al., Anal. Biochem., 2000, 286, 91-98; Larson, et al., Cytometry, 2000, 41, 203-208), subtractive cloning, differential display (DD) (Jurecic and Belmont, Curr. Opin. Microbiol., 2000, 3, 316-21), comparative genomic hybridization (Carulli, et al., J. Cell Biochem. Suppl., 1998, 31, 286-96), FISH (fluorescent in situ hybridization) techniques (Going and Gusterson, Eur. J. Cancer, 1999, 35, 1895-904) and mass spectrometry methods (reviewed in (To, Comb. Chem. High Throughput Screen, 2000, 3, 235-41).

Methods for treating a disease or disorder

In one preferred embodiment of the present invention, the invention comprises a method for treating a disease or disorder in an individual. Said method comprises administering to said individual, in a pharmaceutically acceptable carrier, a sufficient amount of an agent capable of interfering with binding between a receptor of the Vps10p-domain receptor family and a neurotrophin and/or proneurotrophin. By "sufficient amount" herein is meant a dose that produces the therapeutic effects for which it is administered. The exact dose will depend on the disorder to be treated, and will be ascertainable by one skilled in the art using known techniques. In

general, the agent of the present invention is administered to an animal in an amount of from 1 μg/kg to about 100 mg/kg per day. In addition, as is known in the art, adjustments for age as well as the body weight, general health, sex, diet, time of administration, drug interaction and the severity of the disease may be necessary, and will be ascertainable with routine experimentation by those skilled in the art.

Agents of the present invention are believed to be useful in promoting the development, maintenance, or regeneration of neurons in vitro and in vivo, including central (brain and spinal chord), peripheral (sympathetic, parasympathetic, sensory, and enteric neurons), and motor neurons. Accordingly, agents of the present invention may be utilized in methods for the treatment of a variety of neurological diseases and disorders. In a preferred embodiment, the formulations of the present invention are administered to a patient to treat neural disorders. By "neural disorders" herein is meant disorders of the central and/or peripheral nervous system that are associated with neuron degeneration or damage. Specific examples of neural disorders include, but are not limited to, Alzheimer's disease, Parkinson's disease, Huntington's chorea, stroke, ALS, peripheral neuropathies, and other conditions characterized by necrosis or loss of neurons, whether central, peripheral, or motor neurons, in addition to treating damaged nerves due to trauma, bums, kidney dysfunction or injury, pancreatic dysfunction or injury, lung dysfunction or injury, injury to fatty tissue, and the toxic effects of chemotherapeutics used to treat cancer and AIDS. For example, peripheral neuropathies associated with certain conditions, such as neuropathies associated with diabetes, AIDS, or chemotherapy may be treated using the formulations of the present invention.

In various embodiments of the invention, agents are administered to patients in whom the nervous system has been damaged by trauma, surgery, stroke, ischemia, infection, metabolic disease, nutritional deficiency, malignancy, or toxic agents, to promote the survival or growth of neurons, or in whatever conditions are treatable with NGF, NT-3, BDNF or NT4-5. For example, agents of the invention can be used to promote the survival or growth of motor neurons that are damaged by trauma or surgery. Also, agents of the invention can be used to treat motoneuron disorders, such as amyotrophic lateral sclerosis (Lou Gehrig's disease), Bell's palsy, and various conditions involving spinal muscular atrophy, or paralysis. Agents of the present invention can be used to treat human neurodegenerative disorders, such as Alz-

heimer's disease, Parkinson's disease, epilepsy, multiple sclerosis, Huntington's chorea, Down's Syndrome, nerve deafness, and Meniere's disease. Agents of the present invention can be used as cognitive enhancer, to enhance learning particularly in dementias or trauma. Alzheimer's disease, which has been identified by the National Institutes of Aging as accounting for more than 50% of dementia in the elderly, is also the fourth or fifth leading cause of death in Americans over 65 years of age. Four million Americans, 40% of Americans over age 85 (the fastest growing segment of the U.S. population), have Alzheimer's disease. Twenty-five percent of all patients with Parkinson's disease also suffer from Alzheimer's disease-like dementia. And in about 15% of patients with dementia, Alzheimer's disease and multi-infarct dementia coexist. The third most common cause of dementia, after Alzheimer's disease and vascular dementia, is cognitive impairment due to organic brain disease related directly to alcoholism, which occurs in about 10% of alcoholics. However, the most consistent abnormality for Alzheimer's disease, as well as for vascular dementia and cognitive impairment due to organic brain disease related to alcoholism, is the degeneration of the cholinergic system arising from the basal forebrain (BF) to both the codex and hippocampus (Bigl et al. in Brain Cholinergic Systems, M. Steriade and D. Biesold, eds., Oxford University Press, Oxford, pp.364-386 (1990)). And there are a number of other neurotransmitter systems affected by Alzheimer's disease (Davies Med. Res. Rev.3:221 (1983)). However, cognitive impairment, related for example to degeneration of the cholinergic neurotransmitter system, is not limited to individuals suffering from dementia. It has also been seen in otherwise healthy aged adults and rats. Studies that compare the degree of learning impairment with the degree of reduced cortical cerebral blood flow in aged rats show a good correlation (Berman et al. Neurobiol. Aging 9:691 (1988)). In chronic alcoholism the resultant organic brain disease, like Alzheimer's disease and normal aging, is also characterized by diffuse reductions in cortical cerebral blood flow in those brain regions where cholinergic neurons arise (basal forebrain) and to which they project (cerebral cortex) (Lofti et al., Cerebrovasc. and Brain Metab. Rev 1:2 (1989)). Such dementias can be treated by administration of agents of the present invention.

Further, agents of the present invention are preferably used to treat neuropathy, and especially peripheral neuropathy. "Peripheral neuropathy" refers to a disorder affecting the peripheral nervous system, most often manifested as one or a combination

of motor, sensory, sensorimotor, or autonomic neural dysfunction. The wide variety of morphologies exhibited by peripheral neuropathies can each be attributed uniquely to an equally wide number of causes. For example, peripheral neuropathies can be genetically acquired, can result from a systemic disease, or can be induced by a toxic agent. Examples include, but are not limited to, diabetic peripheral neuropathy, distal sensorimotor neuropathy, or autonomic neuropathies such as reduced motility of the gastrointestinal tract or atony of the urinary bladder. Examples of neuropathies associated with systemic disease include post-polio syndrome or AIDS-associated neuropathy; examples of hereditary neuropathies include Charcot-Marie-Tooth disease, Refsum's disease, Abetalipoproteinemia, Tangier disease, Krabbe's disease, Metachromatic leukodystrophy, Down's Syndrome, Fabry's disease, and Dejerine-Sottas syndrome; and examples of neuropathies caused by a toxic agent include those caused by treatment with a chemotherapeutic agent such as vincristine, cisplatin, methotrexate, or 3'-azido-3'-deoxythymidine. Other neural diseases that could benefit from treatment with one or more agents of the present invention include depression and mania.

Accordingly, a method of treating a neural disorder in a mammal comprising administering to the mammal a therapeutically effective amount of one or more agents of the present invention is provided.

Methods of administration

Agents used in the methods of the present invention are generally administered to an animal in the form of a suitable pharmaceutical composition. Accordingly, the present invention also relates to a pharmaceutical composition comprising an agent as defined herein. Such compositions typically contain the agent and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the agent, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of suitable routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL.TM. (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the

injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the agent in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the agent into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatine capsules or compressed into tablets. For the purpose of oral therapeutic administration, the agent can be incorporated with excipients and used in the form of tablets, troches, or capsules, oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatine; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavouring agent such as peppermint, methyl salicylate, or orange flavouring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in

the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The agent can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the agent is prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for deter-

mining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50} /ED_{50}. Agents which exhibit large therapeutic indices are preferred. While agents that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such agents to the site of affected tissue in order to minimize potential damage to other cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any agent used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the $IC_{.50}$ (i.e., the concentration of the test agent which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography. With respect to inhibition of Sortilin 10-20 µmol of Neurotensin is used to inhibit Sortilin in a cell culture.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

The agents of the present invention can further be inserted into vectors and used in gene therapy. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Pat. No 5,328,470) or by stereotactic injection (see e.g., Chen et al. (1994) Proc. Natl. Acad. Sci. USA 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

Vectors suitable for use in gene therapy are known in the art. For example, adenovirus-derived vectors can be used. The genome of an adenovirus can be manipulated such that it encodes and expresses a gene product of interest but is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. See for example Berkner et al. (1988) BioTechniques 6:616; Rosenfeld et al. (1991) Science 252:431-434; and Rosenfeld et al. (1992) Cell 68:143-155. Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 dl324 or other strains of adenovirus (e.g., Ad2, Ad3, Ad7 etc.) are well known to those skilled in the art. Recombinant adenoviruses can be advantageous in certain circumstances in that they are not capable of infecting nondividing cells. Furthermore, the virus particle is relatively stable and amenable to purification and concentration, and as above, can be modified so as to affect the spectrum of infectivity. Additionally, introduced adenoviral DNA (and foreign DNA contained therein) is not integrated into the genome of a host cell but remains episomal, thereby avoiding potential problems that can occur as a result of insertional mutagenesis in situations where introduced DNA becomes integrated into the host genome (e.g., retroviral DNA). Moreover, the carrying capacity of the adenoviral genome for foreign DNA is large (up to 8 kilobases) relative to other gene delivery vectors (Berkner et al. cited supra; Haj-Ahmand and Graham (1986) J. Virol. 57:267). Most replication-defective adenoviral vectors currently in use and therefore favoured by the present invention are deleted for all or parts of the viral E1 and E3 genes but retain as much as 80% of the adenoviral genetic material (see, e.g., Jones et al. (1979) Cell 16:683; Berkner et al., supra; and Graham et al. in Methods in Molecular Biology, E. J. Murray, Ed. (Humana, Clifton, N.J., 1991) vol. 7, pp. 109-127). Expression of the gene of interest comprised in the nucleic acid molecule can be under control of, for example, the E1A promoter, the major late promoter (MLP) and associated leader sequences, the E3 promoter, or exogenously added promoter sequences.

Yet another viral vector system useful for delivery of the agents of the invention is the adeno-associated virus (AAV). Adeno-associated virus is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle. (For a review see Muzyczka et al. Curr. Topics in Micro. and Immunol. (1992) 158:97-129). Adeno-associated viruses exhibit a high frequency of stable integration (see for ex-

ample Flotte et al. (1992) Am. J. Respir. Cell. Mol. Biol. 7:349-356; Samulski et al. (1989) J. Virol. 63:3822-3828; and McLaughlin et al. (1989) J. Virol. 62:1963-1973). Vectors containing as few as 300 base pairs of AAV can be packaged and can integrate. Space for exogenous DNA is limited to about 4.5 kb. An AAV vector such as that described in Tratschin et al. (1985) Mol. Cell. Biol. 5:3251-3260 can be used to introduce DNA into T cells. A variety of nucleic acids have been introduced into different cell types using AAV vectors (see for example Hermonat et al. (1984) Proc. Natl. Acad. Sci. USA 81:6466-6470; Tratschin et al. (1985) Mol. Cell. Biol. 4:2072-2081; Wondisford et al. (1988) Mol. Endocrinol. 2:32-39; Tratschin et al. (1984) J. Virol. 51:611-619; and Flotte et al. (1993) J. Biol. Chem. 268:3781-3790). Other viral vector systems that may be useful for delivery of the agents of the invention are derived from herpes virus, vaccinia virus, and several RNA viruses.

It should be understood that such treatments may also comprise administration of more than one agent, in which case the agents may be either administered concurrently and/or separately.

Animals

In one embodiment of the present invention, agents capable of modulating the activity of a neurotrophin and/or pro-neurotrophin are administered to an animal. Said animal is preferably any animal that expresses a protein of the neurotrophin family, more preferably a mammal, more preferably a domestic animal and most preferably a human being.

Methods for screening for a compound which alters the binding of at least one neurotrophin and/or a pro-neurotrophin to a receptor of the Vps10p-domain receptor family

In one preferred embodiment of the present invention, the invention comprises an in vitro method for screening for a compound which alters the binding of at least one neurotrophin and/or a pro-neurotrophin to a receptor of the Vps10p-domain receptor family, said method preferably comprising the steps of:

a) providing an assay for measuring the binding of a neurotrophin and/or a pro-neurotrophin to a receptor of the Vps10p-domain receptor family

b) adding the compound to be tested to the assay, and

c) determining the amount of a neurotrophin and/or a pro-neurotrophin bound to the receptor of the Vps10p-domain receptor family, and

d) comparing the amount determined in step c) with an amount measured in the absence of the compound to be tested,

e) wherein a difference in the two amounts identifies a compound which alters the binding of neurotrophins and/or pro-neurotrophins to the receptor of the Vps10p-domain receptor family.

In one preferred embodiment of this screening method of the present invention, the neurotrophin is selected from NGF, BDNF, NT-3 or NT-4/5. Even more preferably, the neurotrophin is NGF or BDNF. The pro-neurotrophin may be selected from pro-NGF, pro-BDNF, pro-NT-3 or pro-NT-4/5. More preferably, the pro-neurotrophin is pro-NGF or pro-BDNF. In one preferred embodiment of this screening method, the receptor is selected from SorLa, Sortilin, SorCS1, SorCS3, or SorCS2. Even more preferably, the receptor is Sortilin. In another embodiment of the screening method of the present invention, the neurotrophin and/or pro-neurotrophin is capable of binding to an extracellular part of the receptor. The receptor may in one embodiment of the present invention be a receptor that is expressed in a cell, within the plasma membrane and/or presented on a plasma membrane. The cell used in the screening method of the present invention may preferably be selected from primary cultures of neuronal cells, neurone-derived cell-lines, transfected cells capable of expressing receptor of the Vps10p-domain receptor family, peripheral neurons and central neurons. Preferably the cells are immortalised cell lines.

Assays that can be used for measuring the binding of a neurotrophin and/or a pro-neurotrophin to a receptor of the Vps10p-domain receptor family are well–known to those skilled in the art and include, but are not restricted to, yeast two-hybrid assays, competitive binding methods, such as RIAs, ELISAs, and the like. Other

tests are Fluorescence resonance energy transfer (FRET), Surface plasmon resonance (Biacore), Western blotting, immunohistochemistry. Results from binding studies can be analyzed using any conventional graphical representation of the binding data, such as Scatchard analysis (Scatchard, Ann. NY Acad. Sci., 51:660-672 [1949]; Goodwin et al., Cell, 73:447-456 [1993]), and the like.

A method for determining the effect of an agent on activity of neurotrophins and/or pro-neurotrophins in cells presenting a receptor of the Vps10p-domain receptor family

In another embodiment of the present invention, a method is provided for determining the effect of an agent on activity of neurotrophins and/or pro-neurotrophins in cells presenting a receptor of the Vps10p-domain receptor family. Said method comprises the steps of:

b) administering said agent to a mammal expressing the receptor,

c) measuring the activity of neurotrophins and/or pro-neurotrophins in said mammal,

d) comparing the measurement of step b) with a measurement obtained in the absence of the compound to be tested,

e) wherein the difference in the two measurements identifies the effect of said agent on the activity of neurotrophins on cells presenting receptors of the Vps10p-domain receptor family.

The mammal may express the receptor naturally or may be transfected with the wild-type receptor gene.

The activity of said neurotrophin and/or pro-neurotrophins in said mammal may be measured by one or more of the following measurements:

a) measuring expression level of a neurotrophin responsive target gene, such as mRNA or protein in tissues of the mammal,

b) measuring expression level of a receptor as defined herein, such as mRNA or protein in tissues of the mammal
c) measuring receptor-mediated binding or transport of neurotrophins and/or pro-neurotrophins bound to the receptor,
d) measuring uptake of neurotrophins and/or pro-neurotrophins into cells of said mammal,
e) measuring signal transduction from said receptor or a related receptor in cells of said mammal,

The related receptor may be p75 receptor or TrkA receptor.

In a preferred embodiment of said method, the method further comprises administering said agent to a mammal lacking expression of said receptor. Said mammal lacking expression of said receptor may only lack expression of said receptor in one or more selected tissues, and/or may have a lowered expression level of said receptor.

Methods for measuring expression of receptor mRNA or protein in tissues of the mammal are well known to those skilled in the art and have been described earlier. Methods for measuring receptor-mediated binding or transport of neurotrophins and/or pro-neurotrophins bound to the receptor are also well-known to those skilled in the art: said methods include, but are not restricted to, yeast two-hybrid screening, Biacore RTM screening, UV cross-linking, and immunoprecipitation.

Methods for measuring the uptake of neurotrophins and/or pro-neurotrophins into cells of a mammal are also well known to those skilled in the art: said methods include but are not restricted to a method wherein neurotrophin/proneurotrophin uptake is measured in cells presenting the receptor and cells not representing the receptor. The neurotrophin/proneurotrophin is preferably labelled, such as labelled radioactively or fluorescently.

A method for modulating the transport of at least one neurotrophin and/or proneurotrophin in or into a neuron of an animal

In another embodiment of the present invention, a method is provided for modulating the transport of at least one neurotrophin and/or pro-neurotrophin out of, or into a cell line or neuron of an animal, said method comprising administering to said animal a sufficient amount of an agent capable of binding a receptor of the Vps10p-domain receptor family. Said modulation may comprise an increase in the anterograde transport of the neurotrophin and/or pro-neurotrophin in the neuron. The modulation may alternatively comprise a decrease in anterograde transport of the neurotrophin and/or pro-neurotrophin in the neuron. In another preferred embodiment, the modulation comprises an increase in the retrograde transport of the neurotrophin and/or pro-neurotrophin in the neuron. In another preferred embodiment, the modulation comprises an decrease in retrograde transport of the neurotrophin and/or pro-neurotrophin in the neuron. The modulation may be conducted by an agent as discussed above.

Soluble receptor

In yet another aspect the invention relates to a soluble receptor of the Vps10p-domain receptor family or a fragment or a variant thereof, said fragment or variant being capable of binding said neurotrophin. In particular the soluble receptor is a soluble Sortilin receptor, or a fragment or a variant thereof. Furthermore, the invention relates to the use of the soluble receptor. For example the soluble receptor may be used for modulating the activity of neurotrophins and/or pro-neurotrophins, used for modulating the activity of other receptors such as p75 and TrkA. In another embodiment the soluble receptor may be used for diagnostic purposes in relation to neurotrophins and pro-neurotrophins, in particular in relation to NGF and proNGF.

In addition thereto the invention relates to the expression of a receptor as defined herein as well as to the isolation and purification thereof, said methods being conducted by standard methods.

Furthermore, the invention relates to a pharmaceutical composition comprising a soluble receptor of the Vps10p-domain receptor family or a fragment or a variant thereof.

Examples

Binding to receptors

All the data provided in Fig. 2-7 are obtained by surface plasmon resonance measurements (BIAcore analysis)

Sequence listing

SEQ ID NO 1: Sortilin sequence

>sp|Q99523|SORT_HUMAN Sortilin precursor (Glycoprotein 95) (Gp95) (Neurotensin receptor 3) (NT3) (100 kDa NT receptor) - Homo sapiens (Human).

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLS***QDRLDAPPPPAAPLPRWSGPIGVSWGL*** ***60***
RAAAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLRGSVSLSWVGDS 120
TGVILVLTTFHVPLVIMTFGQSKLYRSEDYGKNFKDITDLINNTFIRTEFGMAIGPENSG 180
KVVLTAEVSGGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLW 240
VSKNFGGKWEEIHKAVCLAKWGSDNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIG 300
VKIYSFGLGGRFLFASVMADKDTTRRIHVSTDQGDTWSMAQLPSVGQEQFYSILAANDDM 360
VFMHVDEPGDTGFGTIFTSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLS 420
EDNSIQTMITFDQGGRWTHLRKPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLS 480
EPNAVGIVIAHGSVGDAISVMVPDVYISDDGGYSWTKMLEGPHYYTILDSGGIIVAIEHS 540
SRPINVIKFSTDEGQCWQTYTFTRDPIYFTGLASEPGARSMNISIWGFTESFLTSQWVSY 600
TIDFKDILERNCEEKDYTIWLAHSTDPEDYEDGCILGYKEQFLRLRKSSMCQNGRDYVVT 660
KQPSICLCSLEDFLCDFGYYRPENDSKCVEQPELKGHDLEFCLYGREEHLTTNGYRKIPG 720
DKCQGGVNPVREVKDLKKKCTSNFLSPEKQNSKSNSVPIILAIVGLMLVTVVAGVLIVKK 780
YVCGGRFLVHRYSVLQQHAEANGVDGVDALDTASHTNKSGYHDDSDEDLLE

SEQ ID NO 2: SorLA sequence

>sp|Q92673|SORL_HUMAN Sortilin-related receptor precursor (Sorting protein-related receptor containing LDLR class A repeats) (SorLA) (SorLA-1) (Low-density lipoprotein receptor relative with 11 ligand-binding repeats) (LDLR relative with 11 ligand-binding)

MATRSSRRESRLPFLFTLVALLPPGALC***EVWTQRLHGGSAPLPQDRGFLVVQGDPRELRL***
WARGDARGASRADEKPLRRKRSAALQPEPIKVYGQVSLNDSHNQMVVHWAGEKSNVIVAL
ARDSLALARPKSSDVYVSYDYGKSFKKISDKLNFGLGNRSEAVIAQFYHSPADNKRYIFA
DAYAQYLWITFDFCNTLQGFSIPFRAADLLLHSKASNLLLGFDRSHPNKQLWKSDDFGQT
WIMIQEHVKSFSWGIDPYDKPNTIYIERHEPSGYSTVFRSTDFFQSRENQEVILEEVRDF
QLRDKYMFATKVVHLLGSEQQSSVQLWVSFGRKPMRAAQFVTRHPINEYYIADASEDQVF
VCVSHSNNRTNLYISEAEGLKFSLSLENVLYYSPGGAGSDTLVRYFANEPFADFHRVEGL
QGVYIATLINGSMNEENMRSVITFDKGGTWEFLQAPAFTGYGEKINCELSQGCSLHLAQR
LSQLLNLQLRRMPILSKESAPGLIIATGSVGKNLASKTNVYISSSAGARWREALPGPHYY
TWGDHGGIITAIAQGMETNELKYSTNEGETWKTFIFSEKPVFVYGLLTEPGEKSTVFTIF
GSNKENVHSWLILQVNATDALGVPCTENDYKLWSPSDERGNECLLGHKTVFKRRTPHATC
FNGEDFDRPVVVSNCSCTREDYECDFGFKMSEDLSLEVCVPDPEFSGKSYSPPVPCPVGS

TYRRTRGYRKISGDTCSGGDVEARLEGELVPCPLAEENEFILYAVRKSIYRYDLASGATE
QLPLTGLRAAVALDFDYEHNCLYWSDLALDVIQRLCLNGSTGQEVIINSGLETVEALAFE
PLSQLLYWVDAGFKKIEVANPDGDFRLTIVNSSVLDRPRALVLVPQEGVMFWTDWGDLKP
GIYRSNMDGSAAYHLVSEDVKWPNGISVDDQWIYWTDAYLECIERITFSGQQRSVILDNL
PHPYAIAVFKNEIYWDDWSQLSIFRASKYSGSQMEILANQLTGLMDMKIFYKGKNTGSNA
CVPRPCSLLCLPKANNSRSCRCPEDVSSSVLPSGDLMCDCPQGYQLKNNTCVKEENTCLR
NQYRCSNGNCINSIWWCDFDNDCGDMSDERNCPTTICDLDTQFRCQESGTCIPLSYKCDL
EDDCGDNSDESHCEMHQCRSDEYNCSSGMCIRSSWVCDGDNDCRDWSDEANCTAIYHTCE
ASNFQCRNGHCIPQRWACDGDTDCQDGSDEDPVNCEKKCNGFRCPNGTCIPSSKHCDGLR
DCSDGSDEQHCEPLCTHFMDFVCKNRQQCLFHSMVCDGIIQCRDGSDEDAAFAGCSQDPE
FHKVCDEFGFQCQNGVCISLIWKCDGMDDCGDYSDEANCENPTEAPNCSRYFQFRCENGH
CIPNRWKCDRENDCGDWSDEKDCGDSHILPFSTPGPSTCLPNYYRCSSGTCVMDTWVCDG
YRDCADGSDEEACPLLANVTAASTPTQLGRCDRFEFECHQPKTCIPNWKRCDGHQDCQDG
RDEANCPTHSTLTCMSREFQCEDGEACIVLSERCDGFLDCSDESDEKACSDELTVYKVQN
LQWTADFSGDVTLTWMRPKKMPSASCVYNVYYRVVGESIWKTLETHSNKTNTVLKVLKPD
TTYQVKVQVQCLSKAHNTNDFVTLRTPEGLPDAPRNLQLSLPREAEGVIVGHWAPPIHTH
GLIREYIVEYSRSGSKMWASQRAASNFTEIKNLLVNTLYTVRVAAVTSRGIGNWSDSKSI
TTIKGKVIPPPDIHIDSYGENYLSFTLTMESDIKVNGYVVNLFWAFDTHKQERRTLNFRG
SILSHKVGNLTAHTSYEISAWAKTDLGDSPLAFEHVMTRGVRPPAPSLKAKAINQTAVEC
TWTGPRNVVYGIFYATSFLDLYRNPKSLTTSLHNKTVIVSKDEQYLFLVRVVVPYQGPSS
DYVVVKMIPDSRLPPRHLHVVHTGKTSVVIKWESPYDSPDQDLLYAIAVKDLIRKTDRSY
KVKSRNSTVEYTLNKLEPGGKYHIIVQLGNMSKDSSIKITTVSLSAPDALKIITENDHVL
LFWKSLALKEKHFNESRGYEIHMFDSAMNITAYLGNTTDNFFKISNLKMGHNYTFTVQAR
CLFGNQICGEPAILLYDELGSGADASATQAARSTDVAAVVVPILFLILLSLGVGFAILYT
KHRRLQSSFTAFANSHYSSRLGSAIFSSGDDLGEDDEDAPMITGFSDDVPMVIA

SEQ ID NO 3: SorCS1 sequence

>tr|Q8WY21 VPS10 domain receptor SorCS - Homo sapiens (Human).

MGKVGAGGGSQARLSALLAGAGLLILCAPGVCGGGSCCPSPHPSSAPRSAST-
PRGFSHQG RPGRAPATPLPLVVRPLFSVAPGDRALSLERARGTGASMAVAARS-
GRRRRSGADQEKAER GEGASRSPRGVLRDGGQQEPGTRERDPDKATRFR-
MEELRLTSTTFALTGDSAHNQAMVHW SGHNSSVILILTKLY-
DYNLGSITESSLWRSTDYGTTYEKLNDKVGLKTILGYLYVCPTNK RKIMLLTDPEI-
ESSLLISSDEGATYQKYRLNFYIQSLLFHPKQEDWILAYSQDQKLYSSA
EFGRRWQLIQEGVVPNRFYWSVMGSNKEPDLVHLEARTVDGHSHYLTCRM-
QNCTEANRNQ PFPGYIDPDSLIVQDHYVFVQLTSGGRPHYYVSYRRNAFAQMK-
LPKYALPKDMHVISTDE NQVFAAVQEWNQNDTYNLYISDTRGVYFTLALEN-
VQSSRGPEGNIMIDLYEVAGIKGMFL ANK-
KIDYQVKTFITYNKGRDWRLLQAPDTDLRGDPVHCLLPYCSLHLHLKVSENPYTSGI
IASKDTAPSIIVASGNIGSELSDTDISMFVSSDAGNTWRQIFEEEHSVLYLDQGGVL-
VAM KHTSLPIRHLWLSFDEGRSWSKYSFTSIPLFVDGVLGEPGEETLIMTVFGHF-
SHRSEWQL VKVDYKSIFDRRCAEEDYRPWQLHSQGEACIMGAKRIYKKRK-
SERKCMQGKYAGAMESEP CVCTEADFDCDYGYERHSNGQCLPAFWFNPSSL-
SKDCSLGQSYLNSTGYRKVVSNNCTDG VREQYTAKPQKCPGKAPRGLRIV-
TADGKLTAEQGHNVTLMVQLEEGDVQRTLIQVDFGDG
IAVSYVNLSSMEDGIKHVYQNVGIFRVTVQVDNSLGSDSAVLYLHVTCPLEHVHL-
SLPFV TTKNKEVNATAVLWPSQVGTLTYVWWYGNNTEPLITLEGSISFRFTSEG-
MNTITVQVSAG NAILQDTKTIAVYEEFRSLRLSFSPNLDDYNPDIPEWRRDIGR-
VIKKSLVEATGVPGQHI LVAVLPGLPTTAELFVLPYQDPAGENKRSTDDLEQISEL-
LIHTLNQNSVHFELKPGVRVL VHAAHL-

TAAPLVDLTPTHSGSAMLMLLSVVFVGLAVFVIYKFKRRVALPSPPSPSTQPGD
SSLRLQRARHATPPSTPKRGSAGAQYAI

SEQ ID NO 4: SorCS3 sequence

Ab028982 human sortilin 3

Reference from EMBO 20.no.9 p2180-2190, 2001: description of mRNA for brain receptor of the Vps10p family,

MEAARTERPAGRPGAPLVRTGLLLLSTWVLAGAEITWDATGGPG RPAA-
PASRPPALSPLSPRAVASQWPEELASARRAAVLGRRAGPELLPQQGGGRG-
GEMQ VEAGGTSPAGERRGRGIPAPAKLGGARRSRRAQPPITQERGDAWA-
TAPADGSRGSRPL AKGSREEVKAPRAGGSAAEDLRLPSTSFALTGDSAHNQAM-
VHWSGHNSSVILILTKLY DFNLGSVTESSLWRSTDYGTTYEKLNDKVGLKTVL-
SYLYVNPTNKRKIMLLSDPEMES SILISSDEGATYQKYRLTFYIQSLLFHPKQEDW-
VLAYSLDQKLYSSMDFGRRWQLMHE RITPNRFYWSVAGLDKEADLVH-
MEVRTTDGYAHYLTCRIQECAETTRSGPFARSIDIS SLVVQDEYIFIQVTTSGRA-
SYYVSYRREAFAQIKLPKYSLPKDMHIISTDENQVFAAV QEWNQNDTYNLY-
ISDTRGIYFTLAMENIKSSRGLMGNIIIELYEVAGIKGIFLANKKV DDQVKTY-
ITYNKGRDWRLLQAPDVDLRGSPVHCLLPFCSLHLHLQLSENPYSSGRISS
KETAPGLVVATGNIGPELSYTDIGVFISSDGGNTWRQIFDEEYNVWFLDWGGAL-
VAMK HTPLPVRHLWVSFDEGHSWDKYGFTSVPLFVDGALVEAGMETHIMTVF-
GHFSLRSEWQ LVKVDYKSIFSRHCTKEDYQTWHLLNQGEPCVMGERKIFKKRK-
PGAQCALGRDHSGSV VSEPCVCANWDFECDYGYERHGESQCVPAFWYN-
PASPSKDCSLGQSYLNSTGYRRIVS NNCTDGLREKYTA-
KAQMCPGKAPRGLHVVTTDGRLVAEQGHNATFIILMEEGDLQRTN
IQLDFGDGIAVSYANFSPIEDGIKHVYKSAGIFQVTAYAENNLGSDTAVLFLHVVCPV
EHVHLRVPFVAIRNKEVNISAVVWPSQLGTLTYFWWFGNSTKPLITLDSSISFT-
FLAE GTDTITVQVAAGNALIQDTKEIAVHEYFQSQLLSFSPNLDYHNPDIPEWRK-
DIGNVIK RALVKVTSVPEDQILIAVFPGLPTSAELFILPPKNLTERRKGNEG-
DLEQIVETLFNAL NQNLVQFELKPGVQVIVYVTQLTLAPLVDSSAGHSSSAMLM-
LLSVVFVGLAVFLIYKF KRKIPWINIYAQVQHDKEQEMIGSVSQSENAPKITLSD-
FTEPEELLDKELDTRVIGGI ATIANSESTKEIPNCTSV

SEQ ID NO 5: SorCS2 sequence

Ab037750 human SorCS2/sortilin 4

Reference from EMBO 20.no.9 p2180-2190, 2001: description of mRNA for brain receptor of the Vps10p family, related to sortilin and SorLa

LIFHPKEEDKVLAYTKESKLYVSSDLGKKWTLLQERVTKDHVFW
SVSGVDADPDLVHVEAQDLGGDFRYVTCAIHNCSEKMLTAPFAGPIDHGSLT-
VQDDYI FFKATSANQTKYYVSYRRNEFVLMKLPKYALPKDLQIIST-
DESQVFVAVQEWYQMDTY NLYQSDPRGVRY-
ALVLQDVRSSRQAEESVLIDILEVRGVKGVFLANQKIDGKVMTLIT
YNKGRDWDYLRPPSMDMNGKPTNCKPPDCHLHLHLRWADNPYVSGTVHTKD-
TAPGLIM GAGNLGSQLVEYKEEMYITSDCGHTWRQVFEEEHHILYLDHGG-
VIVAIKDTSIPLKIL KFSVDEGLTWSTHNFTSTSVFVDGLLSEPGDETLVMTVF-

GHISFRSDWELVKVDFRPS FSRQCGEEDYSSWELSNLQGDRCIMGQQRSFRK-
RKSTSWCIKGRSFTSALTSRVCECR DSDFLCDYG-
FERSPSSESSTNKCSANFWFNPLSPPDDCALGQTYTSSLGYRKVVSNVC
EGGVDMQQSQVQLQCPLTPPRGLQVSIQGEAVAVRPGEDVLFVVRQEQGDVLT-
TKYQV DLGDGFKAMYVNLTLTGEPIRHRYESPGIYRVSVRAENTAGHDEAV-
LFVQVNSPLQAL YLEVVPVIGLNQEVNLTAVLLPLNPNLTVFYWWIGHSLQPLL-
SLDNSVTTRFSDTGDV RVTVQAACGNSVLQDSRVLRVLDQFQVMPLQFSKEL-
DAYNPNTPEWREDVGLVVTRLL SKETSVPQELLVTVVKPGLPTLAD-
LYVLLPPPRPTRKRSLSSDKRLAAIQQVLNAQKI
SFLLRGGVRVLVALRDTGTGAEQLGGGGGYWAVVVLFVIGLFAAGAFILYKFKRK-
RPG RTVYAQMHNEKEQEMTSPVSHSEDVQGAVQGNHSGVVLSINSREMH-
SYLVS

SEQ ID NO 6: NGF sequence

>sp|P01138|NGF_HUMAN Beta-nerve growth factor precursor (Beta-NGF) - Homo sapiens (Human). Signal peptide underlined, propeptide part in bold italics:

MSMLFYTLITAFLIGIQA***EPHSESNVPAGHTIPQVHWTKLQHSLDTALRRARSAPAAAIA***
ARVAGQTRNITVDPRLFKKRRLRSPRVLFSTQPPREAADTQDLDFEVGGAAPFNRTHRSK
RSSSHPIFHRGEFSVCDSVSVWVGDKTTATDIKGKEVMVLGEVNINNSVFKQYFFETKCR
DPNPVDSGCRGIDSKHWNSYCTTTHTFVKALTMDGKQAAWRFIRIDTACVCVLSRKAVRR
A

SEQ ID NO 7: BDNF sequence

>gi|114900|sp|P23560|BDNF_HUMAN Brain-derived neurotrophic factor precursor (BDNF)

MTILFLTMVISYFGCMKA***APMKEANIRGQGGLAYPGVRTHGTLESVNGPKAGSGLTSLAD-***
TFEHVIEEL
LDEDQKVRPNEENNKDADLYTSRVMLSSQVPLEPPLLFLLEEYKNYLDAANMSMRVRRHS-
DPARRGELSV
CDSISEWVTAADKKTAVDMSGGTVTVLEKVPVSKGQLKQYFYETKCNPMGYTKEGCRGID-
KRHWNSQCRT
TQSYVRALTMDSKKRIGWRFIRIDTSCVCTLTIKRGR

SEQ ID NO 8: neurotrophin-3 sequence

>gi|128581|sp|P20783|NT3_HUMAN Neurotrophin-3 precursor (NT-3) (Neurotrophic factor) (HDNF) (Nerve growth factor 2) (NGF-2)
MSILFYVIFLAYLRGI***QGNNMDQRSLPEDSLNSLIIKLIQADILKNKLSKQMVD-***
VKENYQSTLPKAEAPR

EPERGGPAKSAFQPVIAMDTELLRQQRRYNSPRVLLSDSTPLEPPPLYLMEDYVG-SPVVANRTSRRKRYA
EHKSHRGEYSVCDSESLWVTDKSSAIDIRGHQVTVLGEIKTGNSPVKQYFYETR-CKEARPVKNGCRGIDD
KHWNSQCKTSQTYVRALTSENNKLVGWRWIRIDTSCVCALSRKIGRT

SEQ ID NO 9: neurotrophin-4 sequence

>gi|462741|sp|P34130|NT5_HUMAN Neurotrophin-5 precursor (NT-5) (Neutrophic factor 5) (Neurotrophin-4) (NT-4) (Neutrophic factor 4)
MLPLPSCSLPILLLFLLPSVPIES***QPPPSTLPPFLAPEWDLLSPRVVLSRGAPAGP-PLLFLLEAGAFRES***
AGAPANRSRRGVSETAPASRRGELAVCDAVSGWVTDRRTAVDLRGREVEVL-GEVPAAGGSPLRQYFFETR
CKADNAEEGGPGAGGGGCRGVDRRHWVSECKAKQSYVRALTADAQGRVGWRWIRID-TACVCTLLSRTGRA

SEQ ID NO 10: neurotensin sequence

qlyenkprrp yil

SEQ ID NO 11: neuromedin sequence

ipyil

SEQ ID NO 12: Receptor associated peptide (RAP)

1 maprrvrsfl rglpallll lflgpwpaas hggkysrekn qpkpspkres geefrmekln
61 qlwekaqrlh lppvrlaelh adlkiqerde lawkklkldg ldedgekear lirnlnvila
121 kygldgkkda rqvtsnslsg tqedglddpr leklwhkakt sgkfsgeeld klwreflhhk
181 ekvheynvll etlsrteeih envispsdls dikgsvlhsr htelkeklrs inqgldrlrr
241 vshqgystea efeeprvidl wdlaqsanlt dkeleafree lkhfeakiek hnhyqkqlei
301 aheklrhaes vgdgervsrs rekhallegr tkelgytvkk hlqdlsgris rarhnel

SEQ ID NO 13: pro-neurotensin/pro-neuromedin

>gi|2828196|sp|P30990|NEUT_HUMAN Neurotensin/neuromedin N precursor [Contains: Large neuromedin N (NmN-125); Neuromedin N (NmN) (NN); Neurotensin (NT); Tail peptide]

MMAGMKIQLVCMLLLAFSSWSLCSDSEEEMKALEADFLTNMHTSKISKAHVPSWK MTLLNVCSLVNNLNS PAEETGEVHEEELVARRKLPTALDGFSLEAMLTIYQLHKICHSRAFQHWELIQEDIL DTGNDKNGKEEVI KRKIPYILKRQLYENKPRRPYILKRDSYYY